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# Periodontal Tissue Regeneration in Beagle Dogs After Laser Therapy

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***Background and Objective:*** **Class III periodontal furcations still represent a challenge for the periodontist. Aim of this study was to test the effect of $CO_2$ laser on the treatment of class III furcation defects.**
***Study Design/Materials and Method:*** **Class III furcation defects 3 mm deep were surgically induced on mandibular premolars on six male Beagle dogs, for a total of 36 defects. After 6–8 weeks of plaque accumulation, the mean depth was 6.8 mm. Quadrants were randomly assigned to a) $CO_2$ laser therapy (laser), b) Guided Tissue Regeneration (GTR) procedure using Gore-Tex® Membranes, (Gore Tex, Flagstaff, Arizona, USA) and c) Scaling and Root planing (Sc/Rp). $CO_2$ laser beam (El.En®, Florence, Italy) was applied to the root surfaces in defocused pulsed mode at 2W, 1 Hz and a duty cycle of 6%, and on periodontal soft tissues at 13W, 40 Hz, and a duty cycle of 40%. Control quadrants received either GTR procedure or Sc/Rp. Mechanical oral hygiene was provided. At 6 months the animals were sacrificed.**
***Results:*** **The laser group showed new attachment formation averaging 1.9 mm (sd ± 0.5), whereas GTR and Sc/Rp showed 0.2 mm (sd ± 0.4) and 0.2 mm (sd ± 0.5) respectively, being the differences statistically significant between the laser group and both GTR and Sc/Rp groups ($p < 0.005$).**
***Conclusion:*** **$CO_2$ laser treatment of class III furcation induced formation of new periodontal ligament, cementum and bone. Lasers Surg. Med. 21:395–402, 1997.** **© 1997 Wiley-Liss, Inc.**

**Key words: laser; guided tissue regeneration; periodontium**

## INTRODUCTION

The ultimate goal of periodontal therapy is to restore the attachment apparatus, i.e., cementum, ligament, and bone, lost as a result of periodontal disease. Several studies have shown that a significant gain of clinical attachment, regeneration of alveolar supporting bone, new cementum, and new periodontal ligament are achievable results. Different approaches have been tested and advocated, such as root surface conditioning [1] and bone grafts [2].

Regeneration has also been achieved using guided tissue regeneration (GTR) procedures, and studies by Nyman et al. [3,4] and Gottlow et al. [5] have demonstrated that tissue regeneration is an achievable result. Guided tissue regeneration may be achieved by using mechanical barriers,

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such as PTFE non-resorbable membranes or resorbable membranes [6]. In this procedure, the barrier is placed to prevent both ingrowth of gingival epithelium, and to avoid the contact between the soft tissue flap and the root surface.

Results similar to Nyman et al. [3,4] and Gottlow et al. [5] were reported by Becker et al. [7] and Magnusson et al. [8] using bio-resorbable membranes and obtaining variable restoration of lost periodontal tissues. It has been noted that GTR procedures are more effective in vertical rather than horizontal bone loss [9]. Pontoriero et al. [10,11] treating class II and III furcation defects in human mandibular molars with advanced periodontal disease demonstrated high unpredictability and low rate of success specially in class III furcation periodontal defects, and that the size of the defect influences the outcome of wound healing.

Furthermore, Pontoriero et al. [12], in an experimental study in beagle dogs demonstrated that if the furcation defect was associated with circumferential bone loss, or if the defect was 5 mm in apico-coronal direction, new attachment failed to occur. Other regenerative procedures such as root conditioning using repeatedly citric acid [13–15] or tetracyclines [16,17] did provide connective tissue repair.

New methods or techniques in periodontal therapy have been investigated in the recent years, such as growth factors and laser therapy. Lynch et al. [18] in beagle dogs and Rutherford et al. [19] in monkeys demonstrated that platelet-derived growth factor (PDGF) in combination with insulin-like growth factor (IGF-I) provide periodontal tissue regeneration in periodontal lesions in these animals.

$CO_2$laser therapy has been applied in dentistry and oral surgery as a sterilizing tool on both hard and soft tissues [20–22]. The $CO_2$ laser is heavily absorbed by water, with 98% of $CO_2$ laser light absorbed by human tissues. This feature results in rapid cell water vaporization. At a selected power setting, a smaller spot size has a greater power density on the tissues than a larger spot size using a larger spot, therefore, will result in less tissue damage by avoiding warming of root dentin and pulp damage. The $CO_2$ laser beam can sterilize dentin [20,21] and alveolar bone [22] with complete superficial re-crystallization of mineral [23] and tubule occlusion [24,25].

Furthermore, when using $CO_2$ laser light in a defocused mode, it has the advantage to sterilize, vaporize inflamed tissues, and stimulate wound healing [26]. Also, as shown by Kingsbury et al. [27], Braun & Liebow [28], and Liebow & Braun [29], $CO_2$ laser irradiation on vital tissues promotes release of growth factors (GF) which enhance tissue regeneration.

Therefore the aim of this study was to evaluate the effects of $CO_2$ laser therapy on alveolar bone, root cementum, and periodontal ligament cells in Class III furcation defect.

## MATERIALS AND METHODS

The present study was performed on six male Beagle dogs, 18 months old, weighing approximately 12–16 kg, and having a full dentition. The dogs had a full medical check-up and authorization for the experiment was approved by the Animal Studies Review Committee at the State University of New York at Buffalo, NY, USA. Mucoperiostal periodontal flap procedure was performed in each of the lower quadrants and class III furcation defects were surgically produced [10] on the second (P2), third (P3), and the fourth (PR) premolar.

### Study Design

A split mouth design was done. Randomly, one side was assigned to receive $CO_2$ laser therapy (test group) while the contra-lateral received GTR surgical procedure (Gore-Tex® Periodontal Material, Flagstaff, AZ, USA), and/or scaling and root planing (control groups). The experimental teeth were the second, third, and fourth premolars. A total of 36 defects was obtained, 12 for each group.

### Surgical Procedure

A mucoperiostal periodontal flap procedure was performed in each of the lower quadrants and class III furcation defects were surgically induced according to Pontoriero et al. [10] on the second (P2), third (P3), and the fourth (P4) premolar. All surgical procedures were performed under general anesthesia (Surital) and local anesthesia (Lidocaine and Epinephrine 1:50.000). The dogs received preoperative professional tooth cleaning and subgingival scaling with Cavitron (Dentsply, USA). At the same time a sulcular incision and a full thickness periodontal flap was elevated on the right and left side of the mandible, both lingually and buccally, from the mesial of the second premolar (P2) to the distal of the fourth premolar (P4). The alveolar bone was adequately exposed to

have clear visibility and easy access to the furcation areas of the test teeth (P2, P3, and P4).

By means of rotating instruments on a high speed hand piece under constant water irrigation, a class III furcation, 3 mm deep, measured from the highest point of the furcation to the alveolar bone level was produced on all experimental teeth. The interdental bone and the buccal and lingual bone surrounding the roots involved were left intact. Stainless steel wire ligatures were applied at the CEJ level of the teeth, with the excess forming a loop that was pushed into the surgically created defects. A piece of thin foil was packed into the defects. This procedure permitted establishment of plaque maintained periodontal defect. The flaps were repositioned with interrupted silk sutures.

The dogs were put on a soft diet and the ligature was left 6–8 weeks to allow plaque accumulation. Then the ligatures and the tin foil were removed. After removal of the ligatures, the dogs were placed on an oral hygiene regimen with daily brushing for 2 weeks.

At day Zero, a sulcular incision and a full thickness periodontal flap was elevated on the right and left side of the mandible, both lingually and buccally, from the mesial of the second premolar (P2) to the distal of the fourth premolar (P4). At the bottom of the defect, a notch was made on the root surface, facing the furcation. This fixed reference point was considered level 0 from which the expected regenerated tissue would initiate growth.

On the experimental side, $CO_2$ laser therapy (El.En®, Florence, Italy) was applied in a defocused mode on the root surfaces of the 12 experimental teeth as well as on the periodontal soft tissues with different power settings. On root surfaces a $CO_2$ laser beam was applied in defocused fashion (2 mm spot size) in pulsed mode with an average power of 2W, at a frequency of 1 Hz and a duty cycle of 6%. The duty cycle is defined as laser pulse duration divided by the duration of one repetition period, and it is variable between 2% and 40% [26]. The pulse duration was of 60 milliseconds.

Along the periodontal pocket wall, the soft tissues were lased at 13W average power obtained at a frequency of 40 Hz in defocused mode (3 mm spot size) and a duty cycle of 40%. In this case the pulse duration was of 40 milliseconds. Following $CO_2$ laser treatment, both soft and hard tissues were cleaned with an ultrasonic device (Cavitron®) to remove the burnt tissues.

On the control side, PTFE membranes (Gore-Tex®, Flagstaff, AZ, USA) were applied on each of the 12 teeth, covering buccal and lingual aspects of the furcation, and secured with sling suture. On both sides, flaps were coronally repositioned and maintained with silk sutures. The remaining 12 teeth were scaled and root planed with curettes and used as control group.

Individual, site-specific occlusal bite stents were prepared with precision impression material and mounted on Rinn Centric X-ray holder. Periapical radiographs were taken, two for each side of the mandible. The occlusal stent was chemically sterilized and kept for the next radiographic measurements.

The membranes were removed after one month. After 6 months of healing, intraoral radiographs were taken using the customized holder and the animals were sacrificed with an overdose of sodium pentobarbital.

The block sections including the experimental teeth were removed and placed in Karnovsky's fixative solution [30]. After fixation, the crowns were removed to expedite the demineralization process. The block sections were demineralized in Osteodec® (Merck, Hatway, NJ USA). The specimens were subsequently embedded in paraffin. Sections (5 μm thick) were cut in a mesiodistal direction parallel to the long axis of the tooth and stained with hematoxylin and eosin and Masson's trichrome stain for connective tissue. Linear measurements were obtained in the histological samples from the fornix of the furcation to the more apical border of the notch.

## RESULTS

The clinical examination revealed marked soft tissue recession at both lingual and buccal sites in the test and control groups so that the furcations presented "through and through" classified as class III defects. The distance between the notch level and the fornix was 6–7 mm in all three tooth groups (test and controls). Histological findings revealed different patterns between the test group and the other two groups (Tables 1, 2).

### Laser Group

One tooth was excluded from the analysis for technical reasons. The remaining 11 teeth were examined. Table 1 shows that all defects examined gained newly formed cementum with collagen fibers and supporting bone formation above the notch level. As described in Table 1, new at-

**TABLE 1. Regeneration of Cementum and Bone in the Treatment Groups**

| | New cementum | | New bone | |
|---|---|---|---|---|
| Treatment group | (mm) | (sd) | (mm) | (sd) |
| Laser (n = 11) | 1.9 | (±0.4) | 1.1 | (±0.5) |
| Membrane (n = 11) | 0.2 | (±0.4) | 0.7[a] | |
| Scaling/root planing (n = 12) | 0.2 | (±0.5) | 0.2 | (±0.5) |

[a]One defect showed new bone.
Anova statistical analysis (Fisher test and Scheffe-T-test) was performed.
Significant differences were found between the laser group and the membrane and/or scaling/root planing group ($p<0.005$).

**TABLE 2. Percentage of Defect Filled (PDF) Radiographically Assessed**

| | Defect filled | | |
|---|---|---|---|
| Treatment group | % | (sd) | Range |
| Laser (n = 12) | 42.0 | (±12.6) | 22.9–64.0 |
| Membrane (n = 12) | 29.8 | (±19.8) | 0.0–63.6 |
| Scaling/root planing (n = 12) | 28.0 | (±19.8) | 0.0–55.0 |

Significant differences were found between the laser group and the membrane and/or scaling/root planing group ($p < 0.005$).

tachment formation ranged between 1.3 and 2.5 mm from the notch level. The averaged newly formed cementum measured 1.9 mm (sd ± 0.4). The bone was present in different heights and frequently found beyond the notch (Figs. 1–3).

### Control Group

In the GTR treatment group one tooth was excluded from the analysis for technical reasons. Eleven teeth were therefore examined, with only four showing newly formed cementum (mean 0.0 mm, sd ± 0.4) ranging between 0.3 and 1.4 mm. In all the other seven specimens junctional epithelium was present to reach the lowest level of the notch.

### Scaling and Root Planing Group

Only four defects showed regeneration of cementum (mean 0.28 mm, sd ± 0.5) whereas all the other defects showed junctional epithelium until to reach the lowest level of the notch.

### Statistical Analysis

Anova statistical analysis was performed using Fisher test and Scheffe-T-test [31]. Statistically significant difference was found between the laser group and both membrane and scaling and root planing groups ($p < 0.005$) whereas no statistically significant differences were found between the membrane and scaling and the root planing groups.

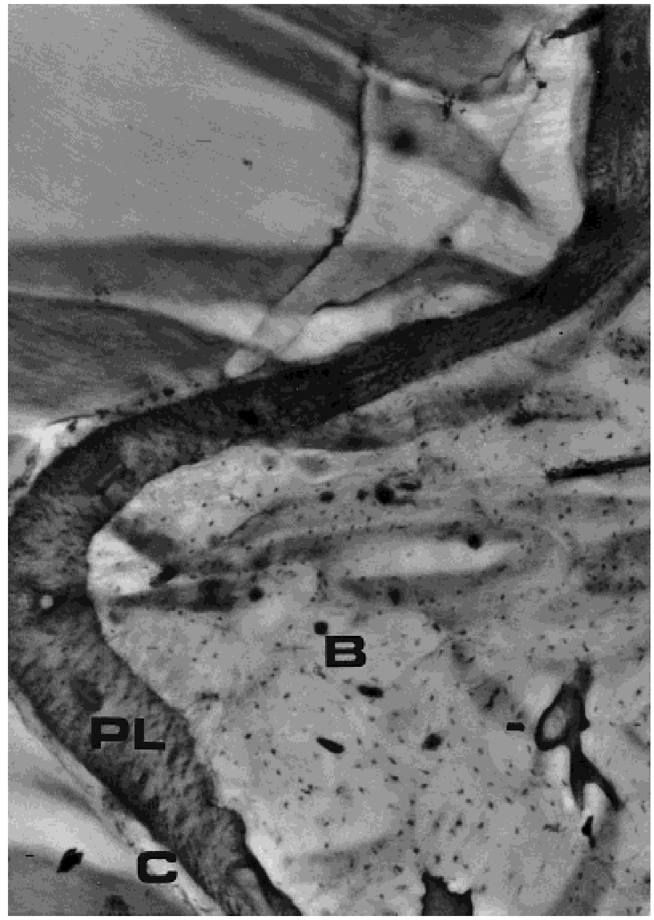


Fig. 1. New cementum (C), periodontal ligament (PL) and bone (B) filling the notch, originally created as reference point. (Hematoxilin & Eosin, 40×)

### Radiographical Analysis

The percentage of defect filled was measured on periapical radiographs by subtracting the whole defect length from the bottom level of the notch to the furcation roof, to the measure from the new bone level to the furcation roof. The data obtained were expressed in percentage of defect filled (PDF), and the data are shown in Table 2.

The laser-treated furcations gave an average PDF of 42.0 (SD ± 12.6), whereas GTR-treated furcation and scaling and root planing gave an average of 29.8 (SD ± 19.8) and 28.0 (SD ± 19.8) respectively. The highest percentage was

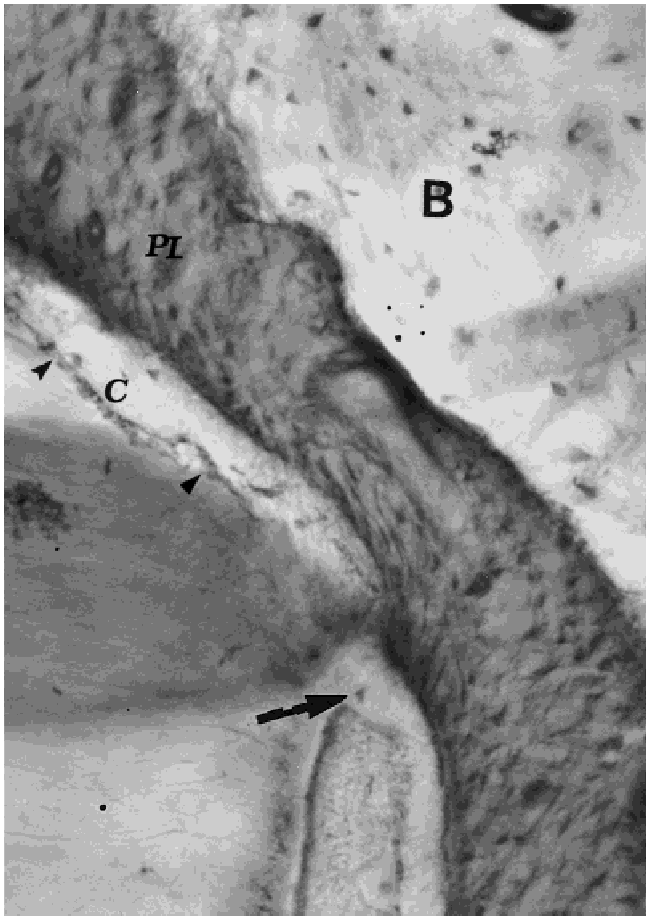


Fig. 2. Higher magnification of periodontium regenerated at the bottom of the notch [bold arrow]. B = bone, PL = periodontal ligament, C = cementum. (Hematoxilin & Eosin, 100×)

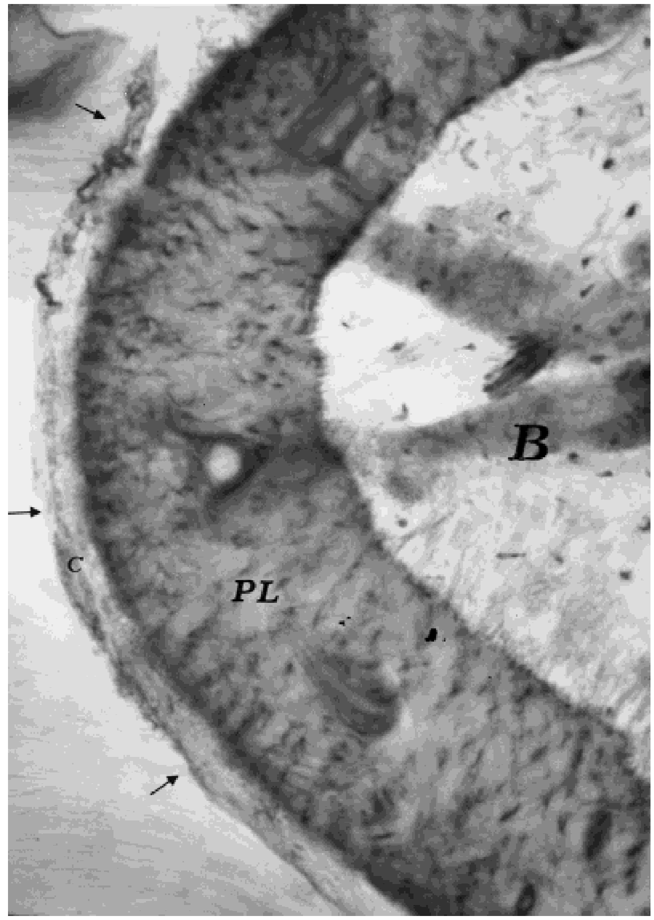


Fig. 3. Higher magnification of periodontium regenerated into the notch. B = bone, PL = periodontal ligament, C = cementum (Hematoxilin & Eosin, 100×)

achieved in the laser group (PDF 64%), whereas zero values were found in both the GTR and the scaling and root planing groups. The minimum value for the laser group was of 22.9. Statistically significant difference ($p < 0.005$) was found between the laser group and GTR and/or Sc/Rp groups.

## DISCUSSION

The results of this study showed the ability of the laser beam to induce tissue regeneration. It also confirmed the incomplete success of GTR therapy to produce new cementum formation in deep defects, as also showed by Pontoriero et al. [12]. Only two specimens provided 1 mm of new cementum formation. This is in agreement with the results of Pontoriero et al. [10]. In the other specimens the dento-gingival epithelium was located in the notch. It seems that the failure of the GTR therapy in treatment of deep furcation defects may be due to the height and size of the defect and flap recession [11] with the involvement in the notch of epithelium.

In the scaling and root planing group, only one specimen demonstrated a small cementum regeneration showing insufficient effect of this technique. Different results were shown by the $CO_2$ laser therapy. In 11 specimens, new cementum formation with new periodontal ligament and bone formation was observed, and was constantly present above the notch.

**Two essential factors emerge from this study.** 1) The height of new cementum formation, periodontal ligament and bone present above the notch with different modes of therapy; 2) The predictability of the results with different modes of therapy.

Also with $CO_2$ laser treatment complete new attachment failed to occur, possibly due to the extent of the furcation defects. However, consistently new attachment formation was observed ranging from 1–2.5 mm.

The results obtained in this study are different from those of Arcoria et al. [32] using a $CO_2$ laser in combination with a Nd:YAG laser to irradiate periodontal tissues in periodontally involved teeth in dogs. The different results may be due to different operative technique and different periods of periodontal wound healing. They vaporized only granulation tissues in periodontal defects with two types of lasers, $CO_2$ and Nd:YAG laser avoiding to treat root surfaces. Histologically wound healing was studied 14 days after laser treatment and no successful results were obtained.

In the present study the $CO_2$ laser was used in a defocused mode both on soft tissue after granulation tissue debridement and on the dentin root surface, at low power settings to maintain pulpal integrity, to sterilize infected tissues, and to stimulate wound healing in periodontal tissues. Although it is difficult to explain these results, they may have occurred because the $CO_2$ laser beam has two main effects on the tissues: first, sterilizing of the tissues and second, biostimulating the wound healing.

Pulpal integrity has been shown to be maintainable if the power of the $CO_2$ laser is within 3W and 1 sec. of pulse duration. Secondary dentin production can also be stimulated, without reaching complete pulp necrosis [33–35].

A $CO_2$ laser in defocused mode (~4 mm spot size) can provide precise surface vaporization and wound sterilization. It is indicated for removal of inflammatory or infectious lesions because the heat of the laser sterilizes both viral and bacterial particles [36]. A defocused beam will perform a bloodless tissue vaporization without exposure to proximal or surrounding structures.

A defocused irradiation at 15–20W with 50–60 Hz gives a precise depth of destruction of soft tissues of the periodontal pocket and allows for the assessment of underlying structures. With a defocused irradiation at 2W with 1 Hz to avoid heating dentin surfaces, the $CO_2$ laser beam can sterilize alveolar bone [22] and infected root surfaces when occluding dentin tubules [21,22, 24,25]. Brugmans et al. [37] showed that very low pulse repetition (5 Hz) should be recommended for surgical procedures where minimal thermal damage is of vital importance to maintain temperature sufficiently low for soft tissues. Complete sterilization of root surfaces and soft tissues of the wall of periodontal pockets may constitute a possible base for tissue regeneration and repair. This idea was demonstrated by Wikesjö et al. [38] who compared, in beagle dogs, the wound healing of experimentally created chronic periodontal defects (chronic defects), and contra-lateral defects that had not been exposed to plaque and calculus (acute defects). Root surfaces of chronic defects presented a less suitable substrate for connective tissue repair than root surfaces of acute defects. Indeed connective tissue repair in the chronic defects was reduced compared to that observed in the acute defects.

Healing in chronic defects with gingival recession, long junctional epithelium, and a smaller amount of connective tissue response was similar to the healing of defects treated with GTR and scaling and root planing in this study. Probably the presence of bacterial cells and endotoxins in the dentinal tubules and in the soft tissues of periodontal pocket walls [39–42] may impair the early healing phase and the adequacy of the inflammatory-reparative response. The wound healing of defects treated with $CO_2$ laser was very similar to the acute defects treated by Wikesjö et al. [38], because the lased tissues, both hard and soft, after laser beam sterilization has a behavior similar to tissues without infections. Therefore it was possible to observe tissue regeneration.

The second beneficial effect of the laser beam on the tissues is the biostimulation in wound healing [26] with an increased healing time and absence of tear and scarring. Liebow & Braun [29] did a series of surgeries using the $CO_2$ laser to remove lesions and left defects uncovered to heal. Defects healed with no evidence of scarring or wound contraction. Defects appeared to reform the original tissues removed. The authors demonstrated histologically the presence of higher levels of Growth Factors, particularly Epidermal Growth Factor and Nerve Growth Factor, in surrounding tissue that may explain better direct specific regenerative activity.

Preliminary clinical data have been reported by Crespi et al. [43], showing successful treatment of periodontal disease on humans.

In conclusion the laser treatment may constitute a reliable tool as antiinfective therapy for periodontal tissues and at the same time provide a biostimulating effect in wound healing to reach a periodontal tissue regeneration both in vertical and horizontal bone loss.

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